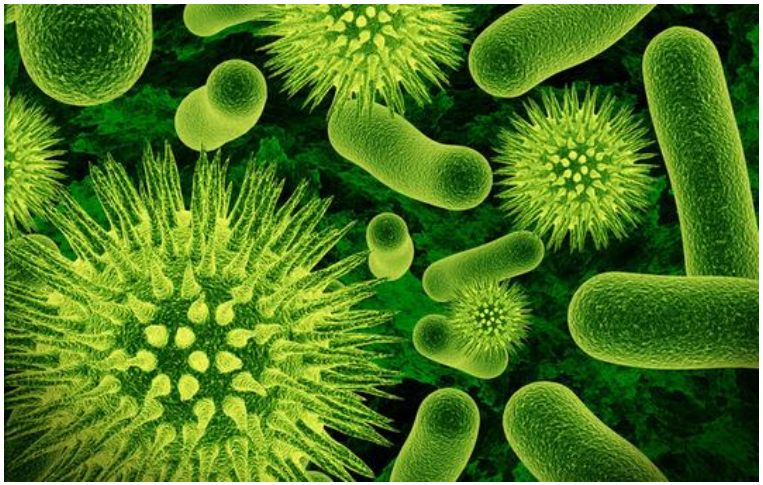
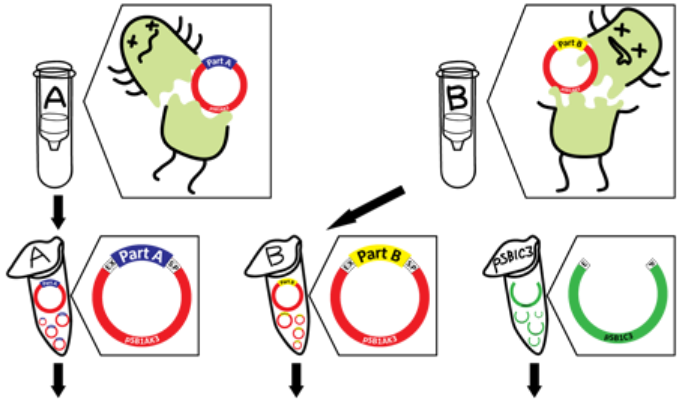
Bacterial Growth and DNA Extraction





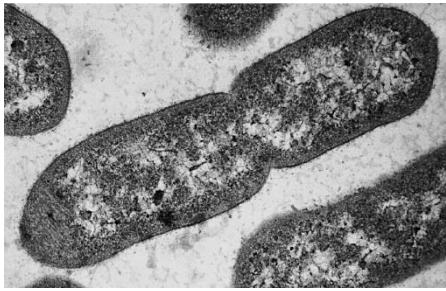
Contents:

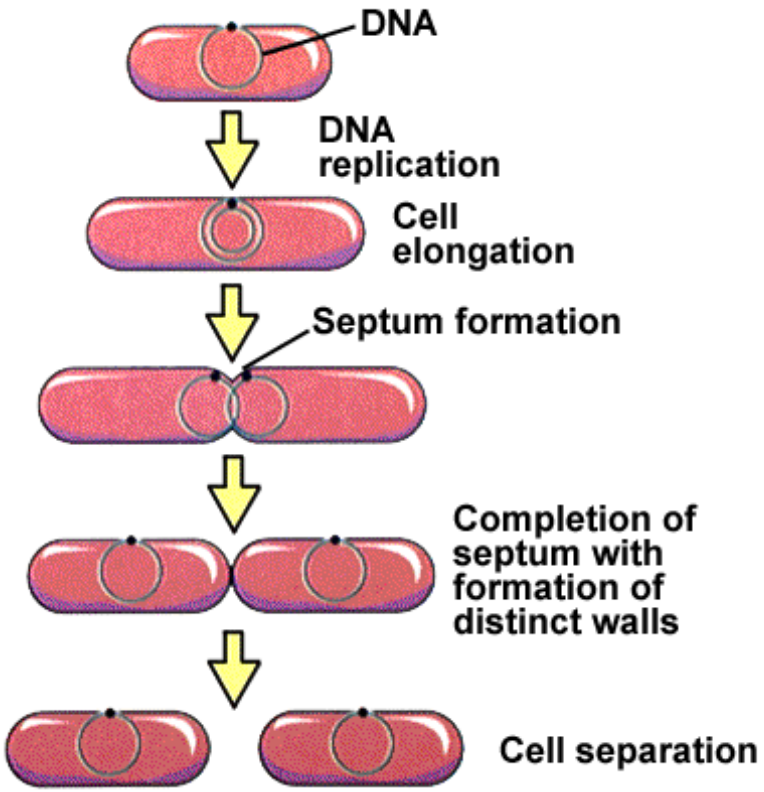
- Stages of bacterial growth
- Bacterial growth curve
- Influence of factors on growth
- Cell concentration measurement
- DNA extraction

Why grow bacteria?

- Bacteria are a basic tool for implementing biotechnological tools:
  - Technological development, such as PCR, which is possible thanks to an enzyme found in hot spring bacteria which can replicate DNA at high temperatures.
  - Use in industry such as cheese-making
  - Identification of diseases such as by using throat cultures
- Known bacteria genomes enable their use to be simple
- Grow quickly and don’t usually require specific conditions and/or tools
- There are many types of bacteria which we don’t know how to crow in the lab

Stages of Microbial Growth

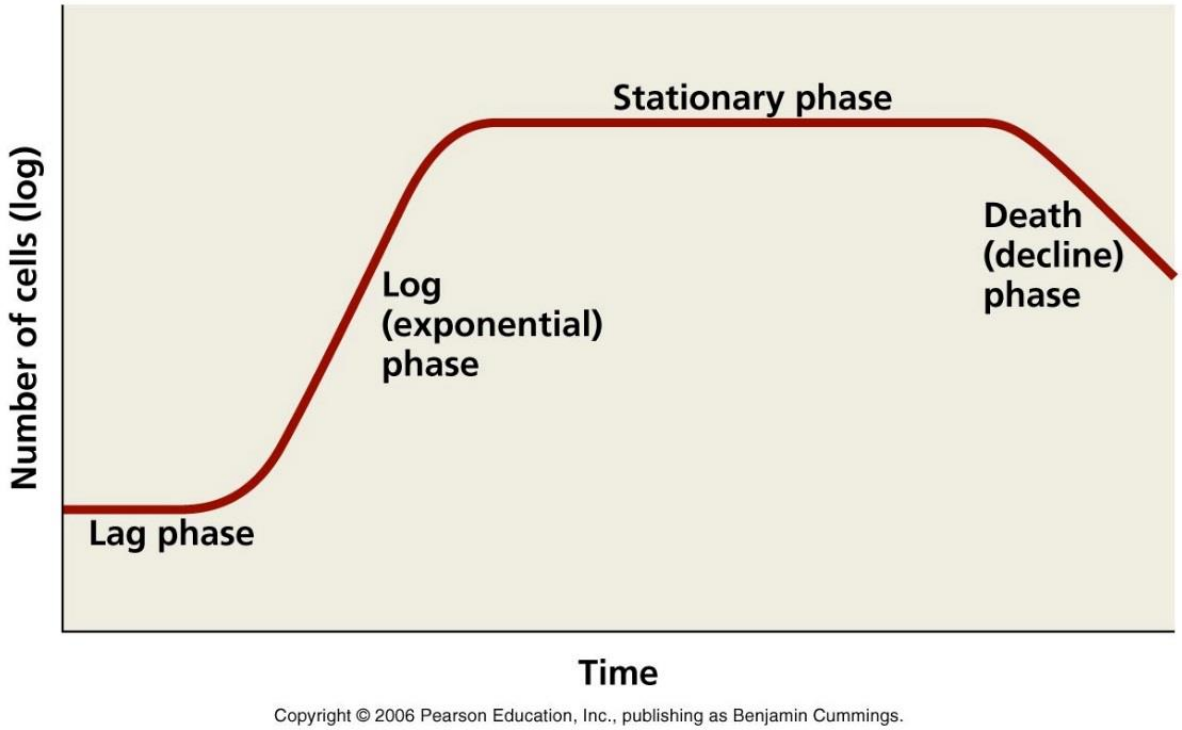




- Bacterial growth mean an increase in their numbers
- Growth occurs with replication of individual cells
- Cells get energy from the medium, increase their volume, and divide after DNA replication to produce 2 daughter cells
- The process takes between 20 minutes and one month (depending on the bacteria and growth conditions)

Stages of Microbial Growth

- The change in the number of bacteria can be seen on the growth curve



<u>Stages:</u>

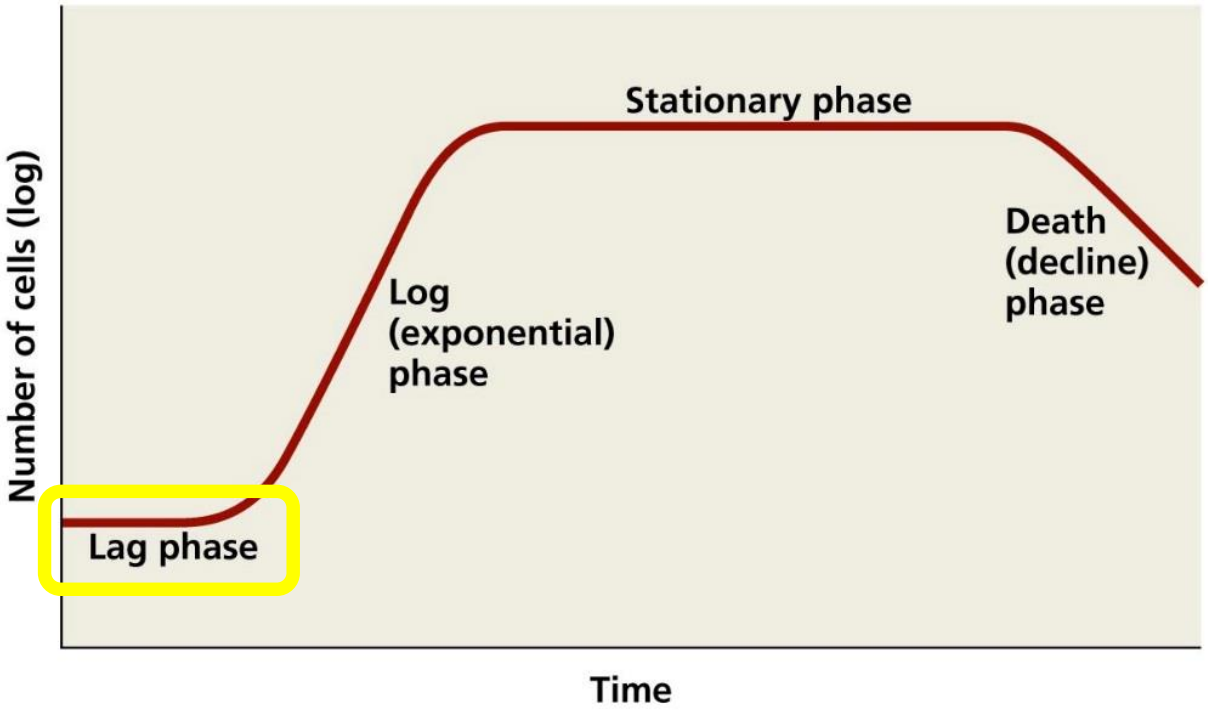
Lag phase

Log phase

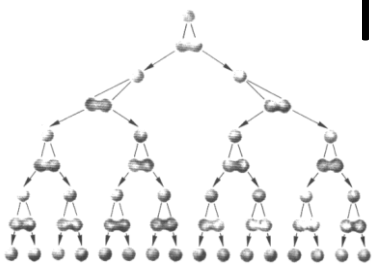
Stationary phase

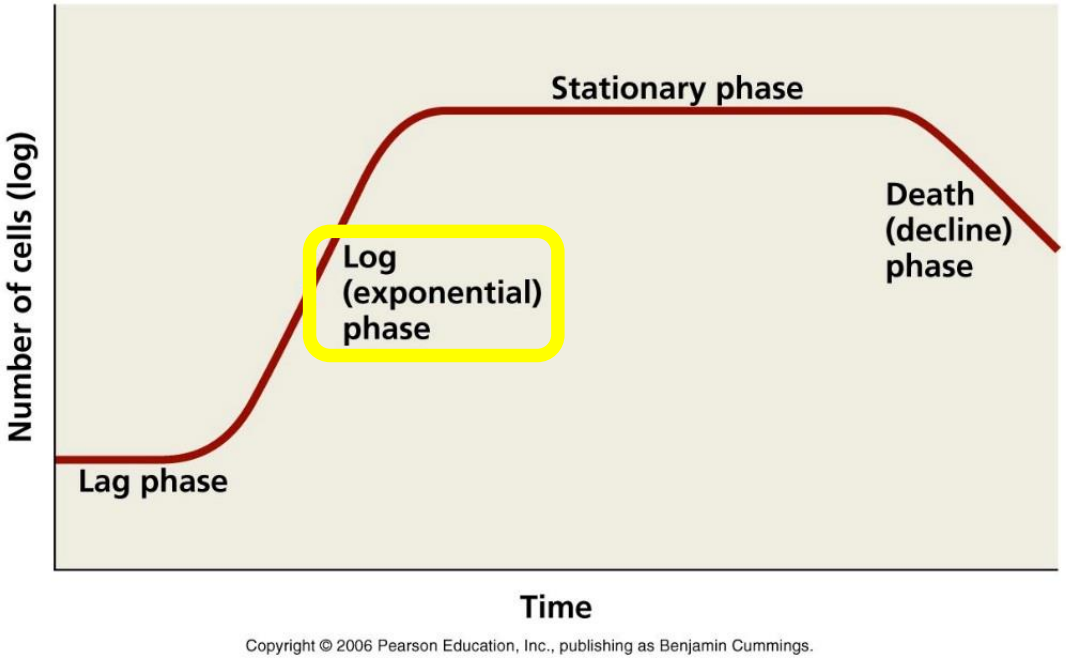
Death phase

The Lag Phase



The Log/Exponential Phase

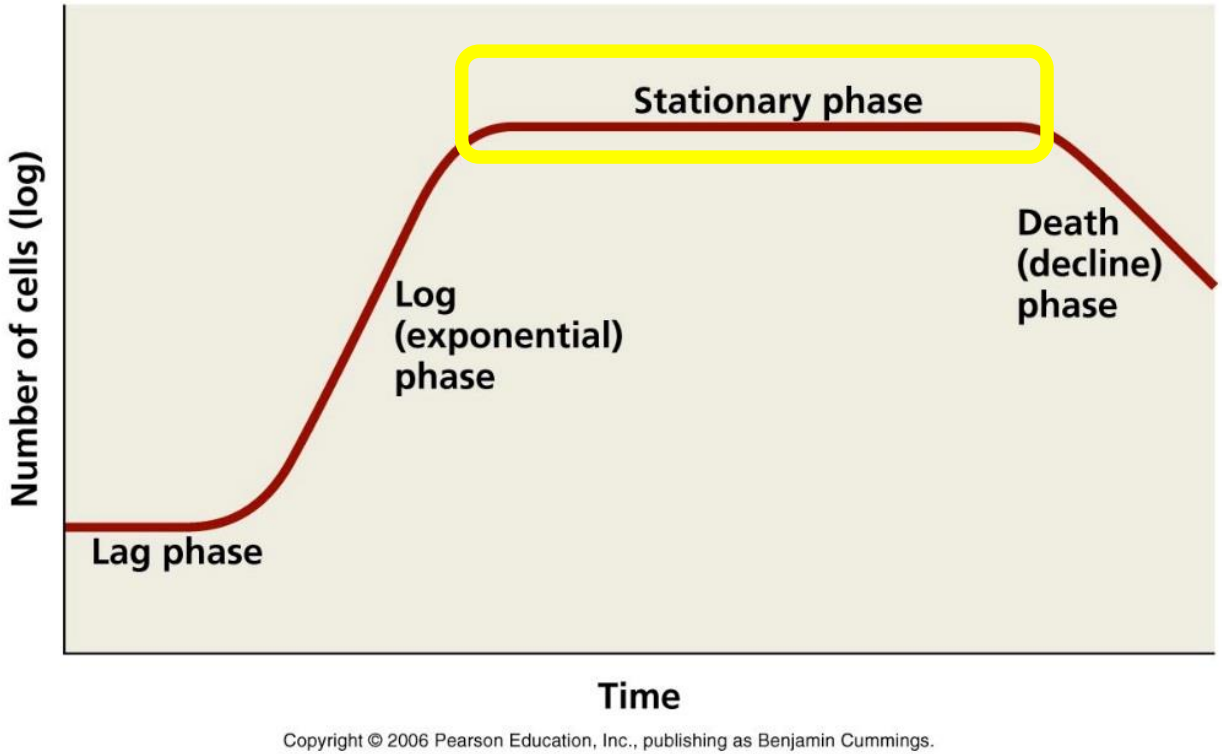




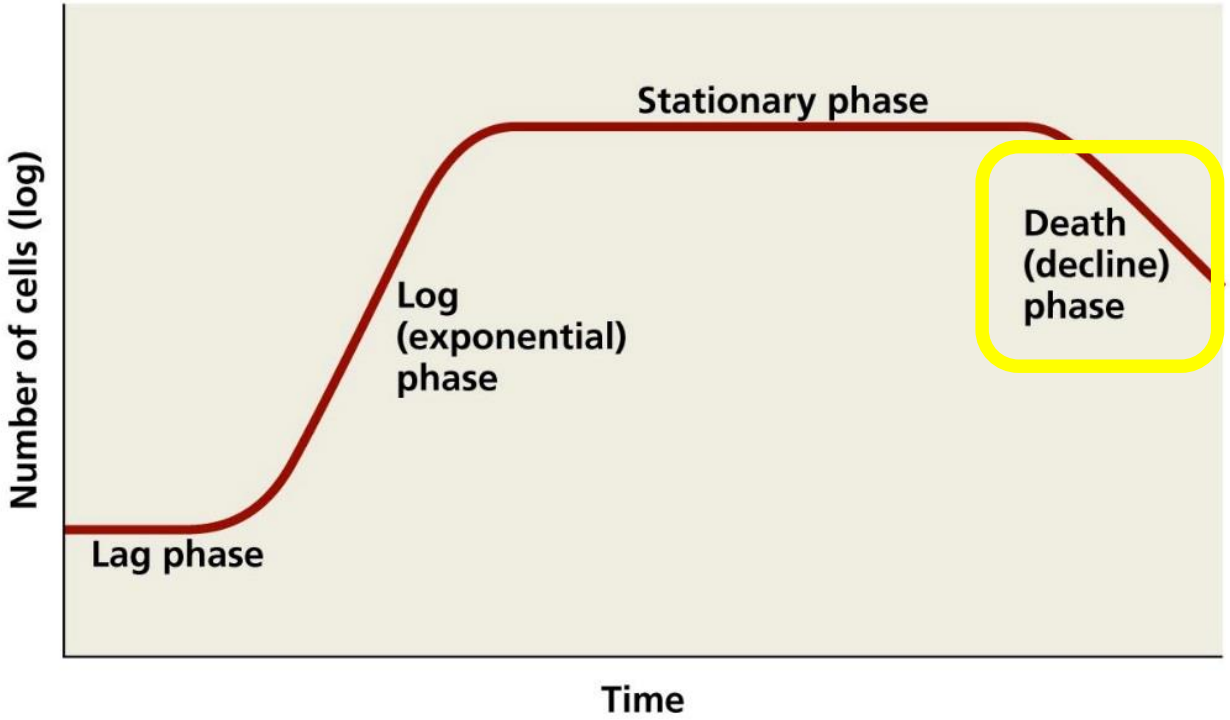
The slope in the rising segment:

$$\frac{\log(2)}{g}$$

Stationary Phase



Death Phase



Relevant equations for the log phase:

- **g**-generation time
- **μ**-growth rate
- **μ**=1/g
- **N**-final # cells
- **n**-# generations
- **N_0**-original # cells
- **T**-growth time

$$N=N_0 2^n$$

$$\log N = \log N_o + n\log 2$$

$$n=(\log N - \log N_o)/\log 2=[\log(N/N_0)]/\log 2$$

$$g=t/n$$

$$t=gn=g\,(\log N - \log N_o)/\log 2 = g\,[\log(N/N_0)]/\log 2$$

$$\log(N/N_0)= (t \log 2)/g$$

$$\text{Log } N = \log N_O + t\,(\log 2)/g$$

Example:

At 6:00, 5,000 bacteria were inoculated in a medium. The lag phase was 2 hours. At 14:30, when the number of cells reached 41 million, the temperature was lowered and the cells were grown until 17:30. At that point, the number of cells was 3.36×10^8. By how much did the growth rate change after the temperature was lowered?

μ_1= **μ_2=**

Solution:

$N_0 = 5 \times 10^3$

$N1 = 41 \times 10^6$

$N2 = 3.36 \times 10^8$

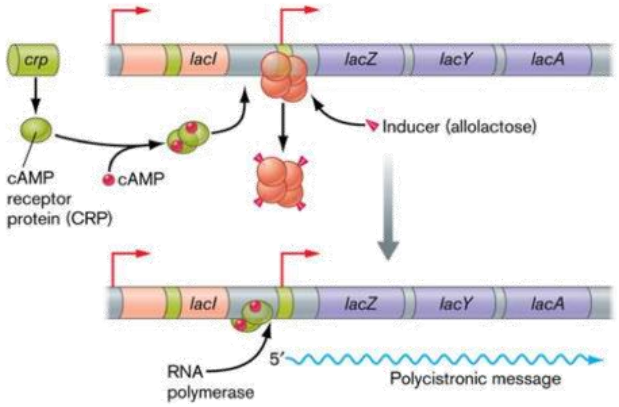
$\log(N/N_0) = (t \log 2)/g$

$t1 = 6.5$ h

$t2 = 3$ h

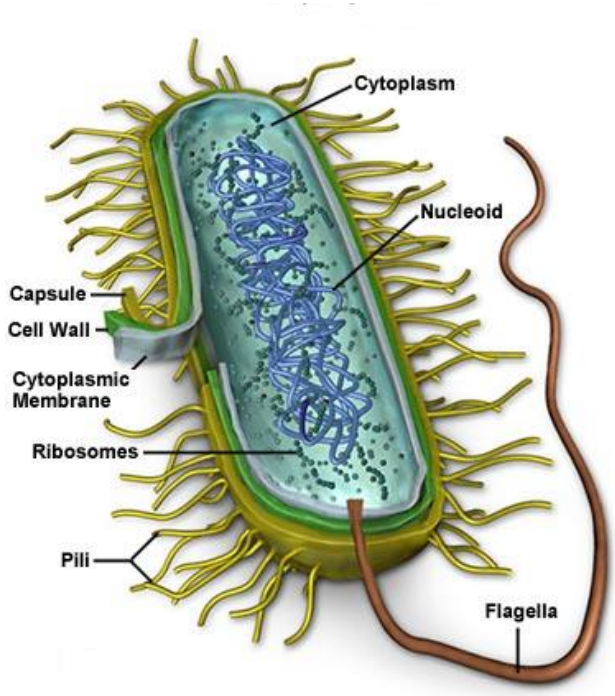
Factors Influencing Growth

- Nutrients- needed for cell growth
- Repressors- inhibit the expression of certain genes
- Inducers- activate gene expression
- Antibiotics- damanges bacterial growth, usually by inhibiting protein synthesis by preventing translation

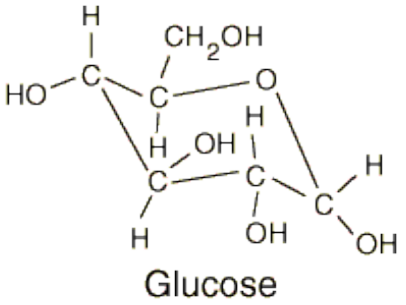


Cell Components

- Water: 80-90% of cell weight
- Dry Cell Make-up
  - 50% carbon
  - 20% oxygen
  - 14% nitrogen
  - 8% hydrogen
  - 3% phosphate
  - 1% sulfur, potassium, and sodium
  - <1% calcium, magnesium, chloride, iron, etc.



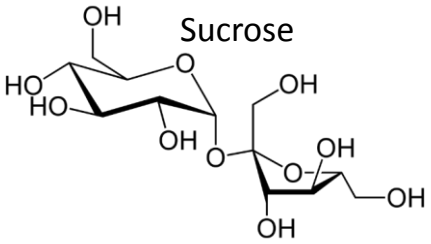
Carbon Sources



Glucose

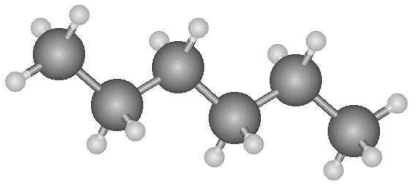
- Carbon is needed in high quantities, as a basic cell building block
- Bacteria usually prefer to use glucose, but in absence of glucose can live on more complex sugars.
- Preference:

  Monosaccharides>Disaccharides>Polysaccharides
- Some bacteria can break down hydrocarbons



Sucrose

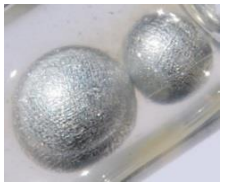
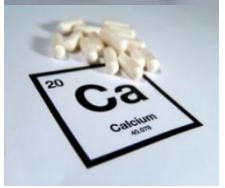
Hexane



Macronutrients

- Nitrogen
- Oxygen
- Phosphate
- Sulfur

Macronutrients

- Sodium – 
- Iron – 
- Potassium – 
- Magnesium – 
- Calcium – 

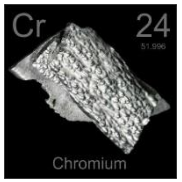
Micronutrients

- Important, but needed in small amounts
- Examples:


selenium

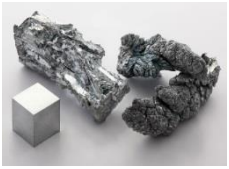

cobalt


copper



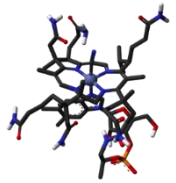
chromium


manganese


zince


nickel

- These metals are usually found in high enough quantities in water

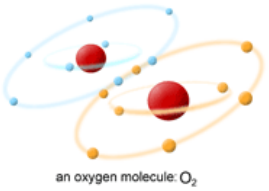

ויטמין B12

- Organic compounds such as vitamins and amino acids

Growth Mediums

- The growth medium provides the necessary nutrients and energy for the bacteria to grow
- There are both liquid and solid mediums
- **Defined medium (synthetic):** contains components with known composition: salts, sugars, alcohols, amino acids, fatty acids, etc.
  - When nutrients are found in low concentrations, the medium is called a poor medium (contrary to a rich medium)
- **Complex Medium (non-synthetic):** contains a mix with an unknown chemical composition: beef extract, yeast extract, blood, etc.

Influence of Oxygen on Growth



Three main groups of bacteria:

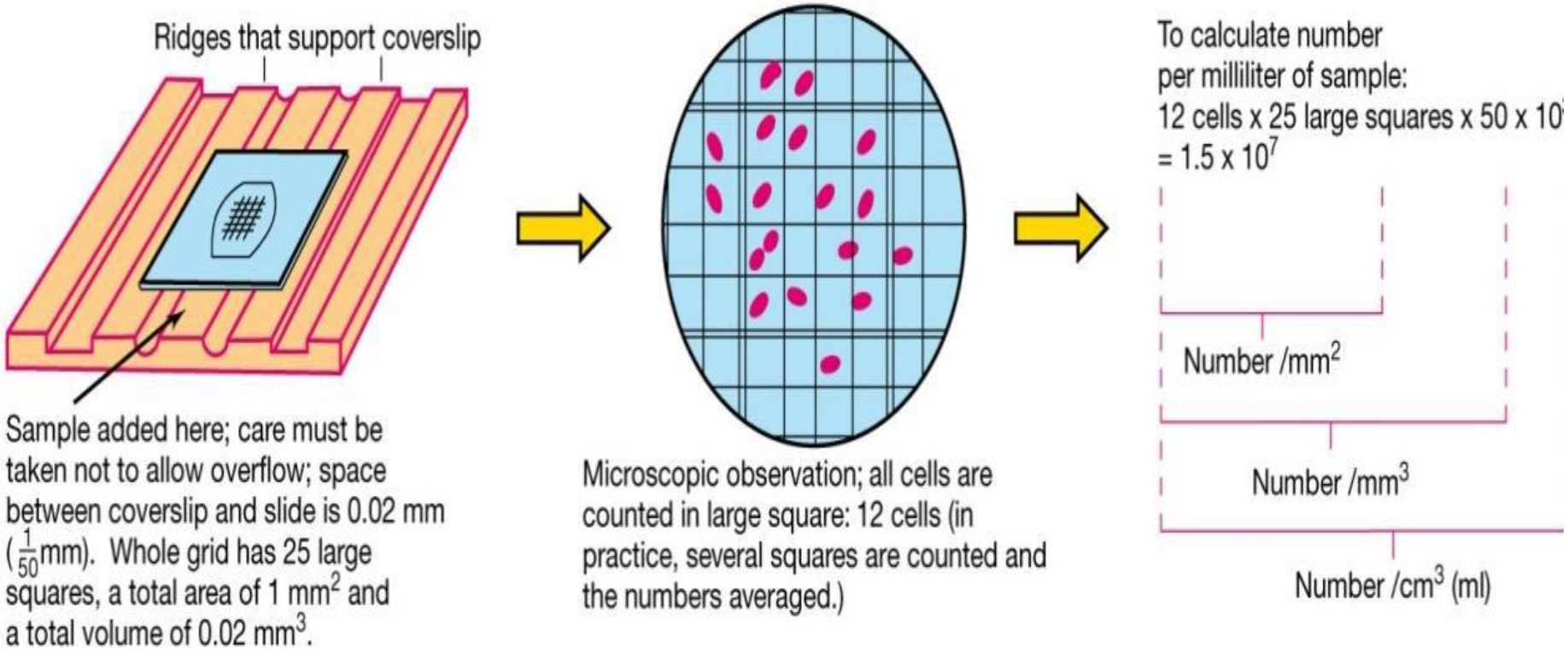
- Obligatory aerobes: need oxygen in order to grow. Oxygen is the final electron receptor in the cell respiration process. Synthesize enzymes to break down metabolic derivatives of oxygen
- Facultative aerobes: grow better in the presence of oxygen, but can grow without it as well
- Obligatory anaerobes: die in presence of oxygen- Sensitive to metabolic derivatives

When oxygen is not the final electron receptor, other elements may be used instead, such as gold, iron, and sulfate **OR** other mechanisms, such as fermentation, may be used

Methods for Measuring Bacterial Concentration

- **Direct-** methods which directly measure the amount of cells
- **Indirect-** methods which measure something which indicates the amount of cells

Direct Methods- Direct Microscopic Count



Pros and Cons

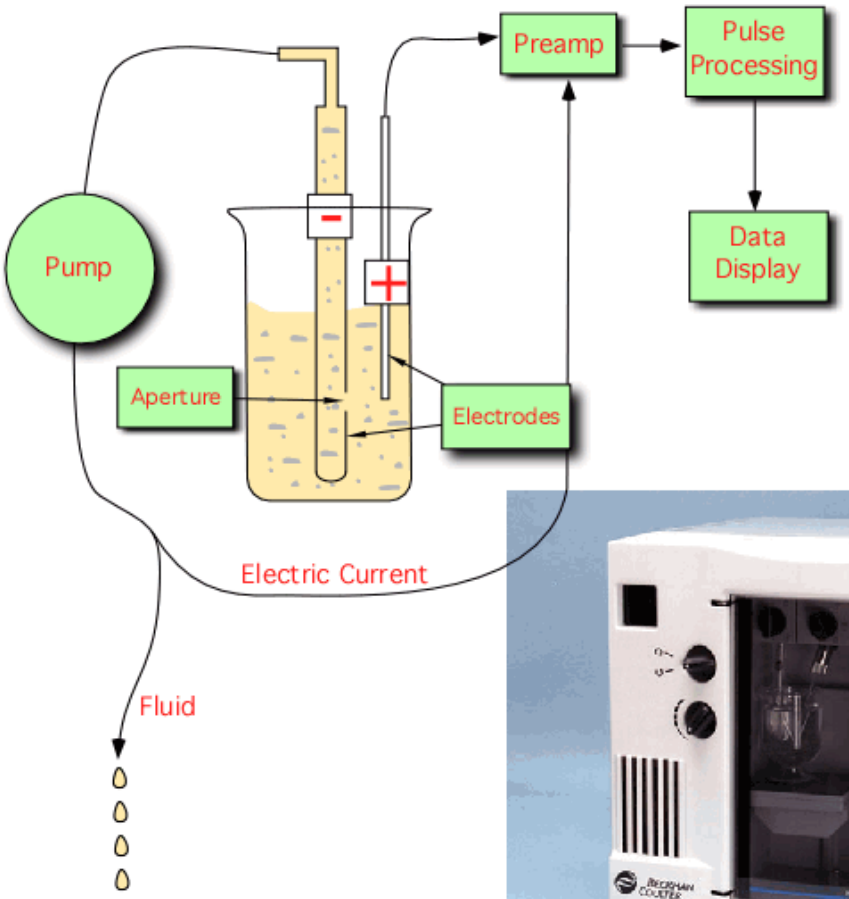
✓**Pros:**

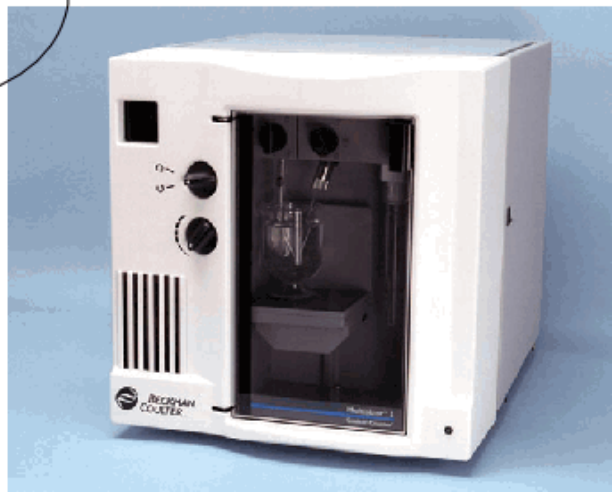
- Relatively quick
- Cheap

×**Cons**

- Impossible to differentiate between live and dead cells
- Hard to identify small cells
- Not sensitive to sample with low concentrations of bacteria
- Hard to reach accuracy
- Work-intensive

Direct Methods- Coulter Counter





Pros and Cons

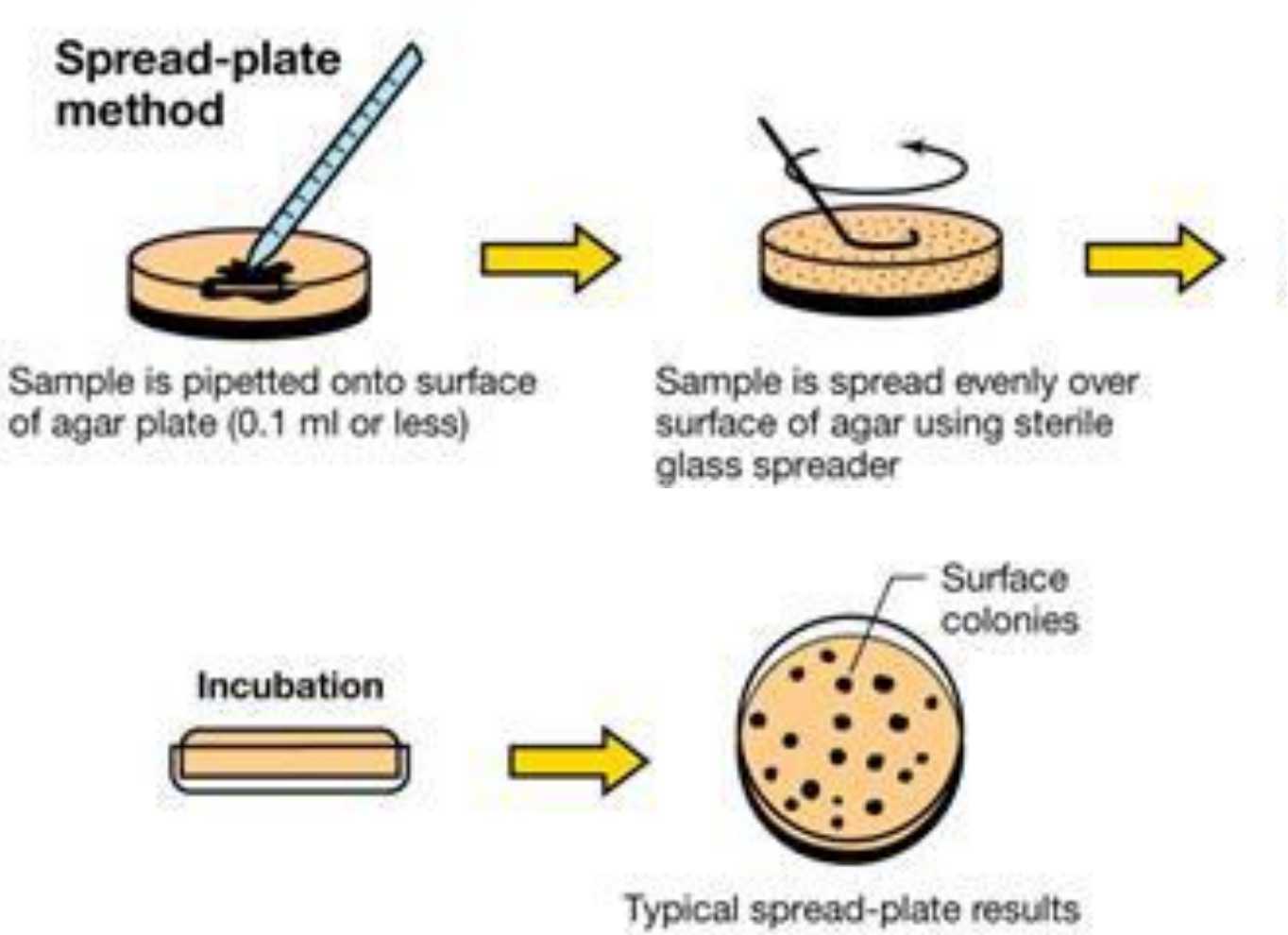
✓**Pros:**

- Relatively easy
- Sensitive
- Accurate

×**Cons**

- Impossible to differentiate between live and dead cells
- Unable to count cells in clusters
- If other small particles are present, they will be counted as cells

Direct Methods- Live Cell Count



Pros and Cons

✓**Pros:**

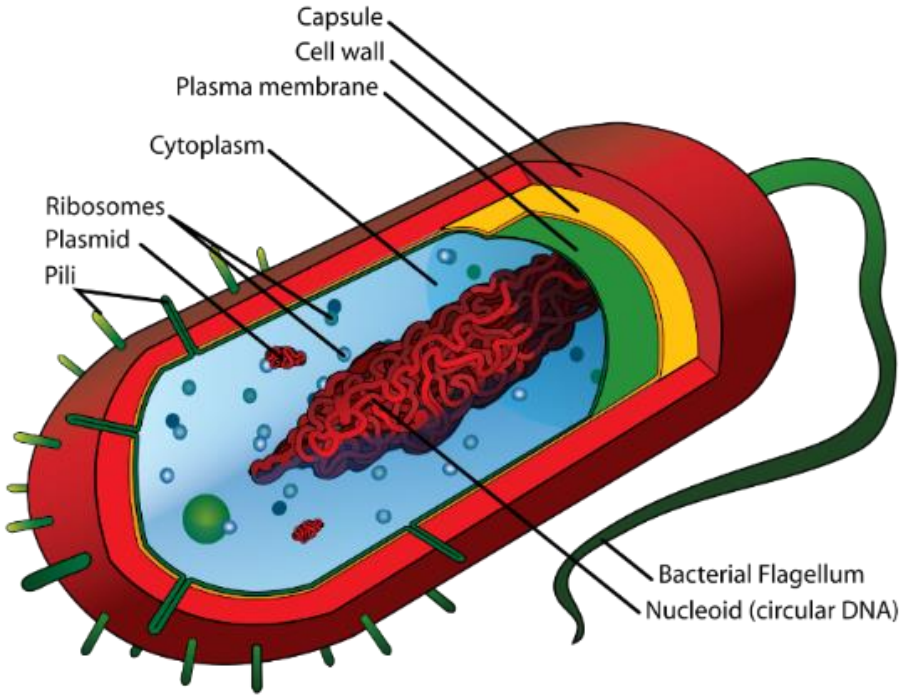
- Easy
- Relatively accurate
- Sensitive for low concentrations

×**Cons**

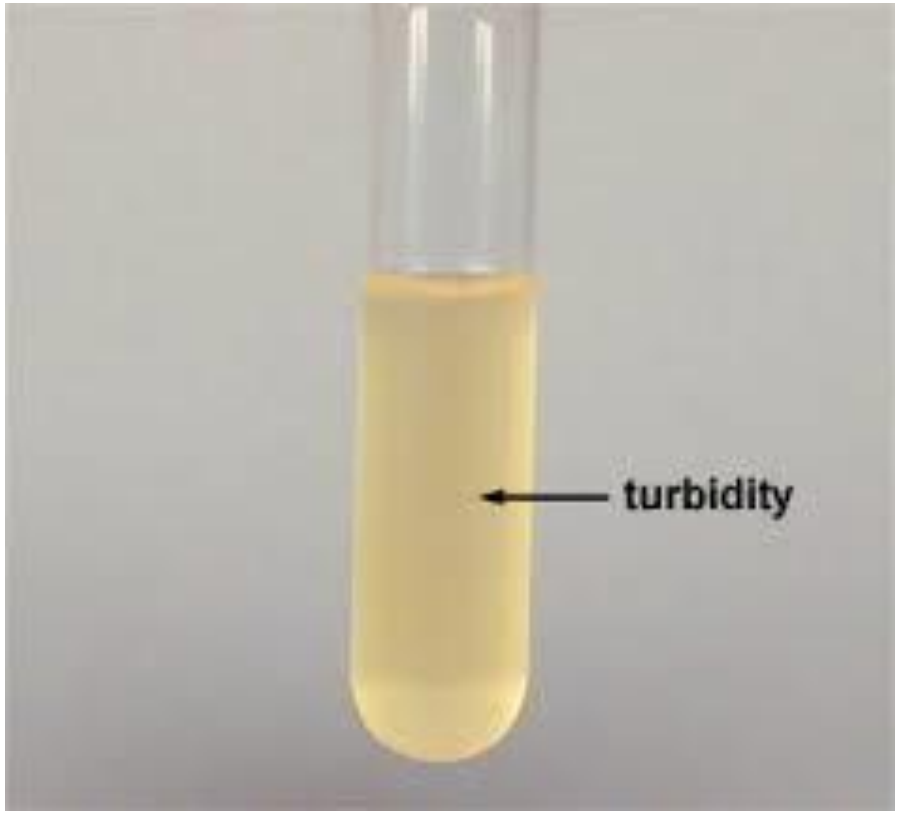
- Since we assume each colony originates from one cell, the concentration units are given in **Colony forming units= CFU**
- Only bacteria which can be grown in the lab will be able to be counted

Indirect Methods- Dry Cell Weight

Indirect Methods- change in cell components



Indirect Methods- optical density



Pros and Cons

✓**Pros:**

- Accurate
- Simple
- Immediate results
- Sample not ruined

×**Cons**

- Both live and dead cells are counted
- Requires high bacteria concentrations (about 10^7 cells/ml but below 10^9 cells/ml)
- Medium must not absorb light

Bacterial DNA Extraction

Preparations

Need high cell concentration to get the most DNA.

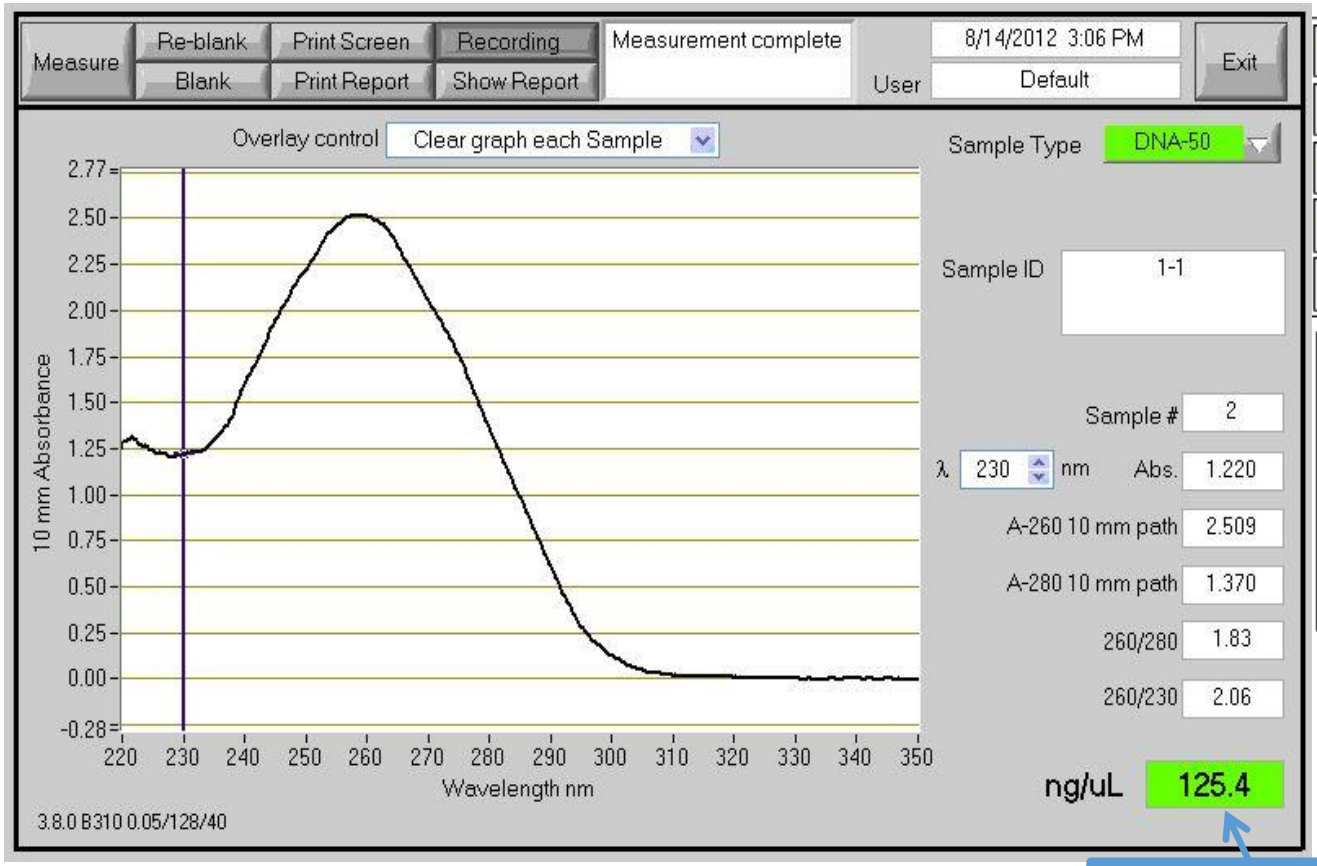
Growth Conditions:

- Grow in 5 ml liquid LB
- Overnight (16-20 hours)
- 37^{o}C

Protocol

1. Harvest cells
2. Resuspend
3. Lyse
4. Neutralize
5. Centrifuge and reserve supernatant
6. Bind DNA to column
7. Wash bound DNA
8. Dry Column
9. Elute DNA

Nanodrop



% protein	% nucleic acid	260:280 ratio
100	0	0.57
95	5	1.06
90	10	1.32
70	30	1.73

% nucleic acid	% protein	260:280 ratio
100	0	2.00
95	5	1.99
90	10	1.98
70	30	1.94

Desired Ratios:

230:260:280=1:1.8:1

Sample Concentration